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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 9/42, A21D 8/04	A1	(11) International Publication Number: WO 96/32472
		(43) International Publication Date: 17 October 1996 (17.10.96)

(21) International Application Number: PCT/DK96/00171

(22) International Filing Date: 11 April 1996 (11.04.96)

(30) Priority Data:
0435/95 11 April 1995 (11.04.95) DK

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: BREAD-IMPROVING ADDITIVE COMPRISING A XYLANOLYTIC ENZYME

(57) Abstract

The present invention relates to bread-improving additives. More specifically, the invention relates to bread-improving additives comprising xylanolytic enzymes derived from strains of *Thermomyces lanuginosus*, to methods for preparing baked products, and to methods for improving the baking properties of flour or dough.

CLAIMS

1. A bread-improving additive, comprising a xylanolytic enzyme derived from a strain of *Thermomyces*.

2. The bread-improving additive according to claim 1, in which the xylanolytic enzyme is derived from a strain of *Thermomyces lanuginosus*.

3. The additive according to either of claims 1-2, in which the xylanolytic enzyme is derived from the strain *Thermomyces lanuginosus* DSM 4109, or a mutant or a variant thereof.

4. The additive according to any of claims 1-3, in which the xylanolytic enzyme has the amino acid sequence presented as SEQ ID NO: 2, or any partial sequence hereof.

5. The additive according to any of claims 1-3, in which the xylanolytic enzyme has an amino acid sequence homologue to the sequence presented as SEQ ID NO: 2.

6. The additive according to any of claims 1-5, in which the xylanolytic enzyme is provided in the form of a monocomponent xylanase preparation.

7. The additive according to claim 6, in which the monocomponent xylanase is

(a) encoded by the DNA sequence presented as SEQ ID NO: 1, or by the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

(b) encoded by a DNA sequence analogue to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyc s cerevisiae* DSM 10133, which analog DNA sequenc ither

(i) is homologous to the xylanas encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

(ii) hybridizes with the same oligonucleotide probe as the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or with the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

(iii) encodes a polypeptide which is at least 70% homologous to the polypeptide encoded by the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

(iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified xylanase derived from the strain *Thermomyces lanuginosus*, DSM 4109, or encoded by the DNA sequence presented as SEQ ID NO: 1, or the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

8. The additive according to any of claims 1-7, in which the xylanolytic enzyme is added in an amount corresponding to of from about 5 to about 5000 FXU/kg of flour, preferably of from about 20 to about 2000 FXU/kg of flour.

9. The additive according to any of claims 1-8, which comprises one or more additional enzymes selected from the group consisting of an amylase, a maltogenase, a lipase, a cellulase, a hemicellulase, a pentosanase, a glucose oxidase, a laccase, a protease and a peroxidase.

10. The additive according to any of claims 1-8, which comprises one or more additional enzymes selected from the group consisting of a lipase, an amylase and an oxidase.

11. The additive according to any of claims 1-8, which comprises one or more additional enzymes selected from the group consisting of an α-amylase and an amyloglucosidase.

12. The additive according to any of claims 1-8, which comprises a lipase.

13. The additive according to any of claims 1-8, which comprises an amylase.

14. The additive according to claim 13, in which the amylase is an α-amylase.

15. The additive according to claim 14, in which the α-amylase is of fungal origin.

16. The additive according to claim 15, in which the α-amylase is derived from a strain of *Aspergillus*.

17. The additive according to claim 16, in which the α-amylase is derived from a strain of *Aspergillus oryzae*.

18. A method of preparing a baked product, which method comprises adding to the flour or to the dough the bread-improving additive according to any of claims 1-17.

19. A method of improving the baking properties of flour and/or dough, which method comprises adding to the flour and/or to the dough the bread-improving additive according to any of claims 1-17.

20. The method according to claim 18, which method comprices adding th bread-improving additive according to any of claims 1-18 to any ingredient of the dough and/or to any mixture of the dough ingredients.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00171

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/42, A21D 8/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, FSTA, WPI, WPIL, CA, US PATENTS FULLTEXT, SCISEARCH, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0507723 A1 (NOVO NORDISK A/S), 7 October 1992 (07.10.92), page 3, line 6 - line 8; page 3, line 25 - line 31; page 3, line 48 - line 49, the claims --	1-20
X	WO 9421785 A1 (NOVO NORDISK A/S), 29 Sept 1994 (29.09.94), page 8, line 12; page 13, line 11 - line 26, claim 15 --	1-20
X	EP 0396162 A1 (UNILEVER NV), 7 November 1990 (07.11.90), page 3, line 3 - line 4, the claims --	1-20

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 1996	18 -07- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Palmcrantz Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00171

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, File 5, Biosis, Dialog accession no. 10989020, Alam M. Gomes et al: "Production and characterization of thermostable xylanases by Thermomyces lanuginosus and Thermoascus aurantiacus grown on lignocelluloses", Enzyme and Microbial Technology 16 (4).1994. 298-302 -- --------	1-20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1 and 7
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

The wording "...mutant or variant thereof" of claim 3 is not considered to be clear and concise since the mutant or variant is not restricted to possess the intended, special features of the parent xylanolytic enzyme.

The wording "homologous" of claim 7 (i) is not considered to be clear and concise since it has not been specified to what extent the sequence is homologous to the xylanase encoding part of the DNA sequence presented as SEQ ID NO:1. It should be clear from the claim that the part(s) of SEQ ID NO:1 that encodes the alleged inventive features of the xylanase is present in the analogue which is defined "homologous".

Thw wording "immunologically reactive with an antibody raised against" of claim 7 (iv) does not define a property that is relevant in the context of the invention, as there is no direct link between the xylanase activity and the immunological features (except for some unknown epitopes of the active site).

Consequently, claims 3 and 7 are not considered to fulfil the requirements of PCT Article 6 regarding clarity and conciseness.